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NORMAL AND MALIGNANT MELANIN-CONTAINING PIGMENT CELLS OF XIPHOPHORINE FISH AS STUDIED WITH FORMALDEHYDE-INDUCED FLUORESCENCE

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Embryonic skin and eyes, and melanomas of xiphophorine fish were investigated by formaldehyde-induced fluorescence in order to test whether the pigment cells in these tissues may be identified by a specific green-yellow fluorescence. Skin of pigmented fish embryos showed no fluorescence in the black pigment cells (melanocytes and melanophores), while skin of albino embryos showed a green-yellow fluorescence in all cells which correspond to the black pigment cells of pigmented embryos. The skin of both pigmented and albino embryos showed a bright organge fluorescence in the red pigment cells (pterinophores). No fluorescence was observed in the retinal pigment epithelium of pigmented embryos, while a green-yellow fluorescence was observed in the pigment epithelium of albino embryos. Neither the melanotic melanomas of pigmented fish nor the amelanotic melanomas of albino fish showed any specific fluorescence.

Since Falck and Rorsman [1], in 1963, observed that treatment with formaldehyde vapor leads to a specific green-yellow fluorescence of melanocytes in human skin, formaldehyde-induced fluorescence microscopy has developed into a valuable method for the identification of melanin-producing cells in normal and neoplastic tissues of man [2–8]. Melanocytes which have been activated for melanin synthesis in vivo [9–11] or which have been induced to differentiate in vitro [12] show an increased intensity of formaldehyde-induced fluorescence. It has been stated, therefore, that a correlation exists between the formation of fluorescent substances and an accumulation of dopa or other melanin precursors due to the activity of the melanin-synthesizing enzyme tyrosinase [2,3,9]. However, the melanocytes of heavily pigmented tissues, such as Negro skin [13] and melanotic melanomas of man [4], show no or only weak fluorescence, and melanomas of hamster and mouse show no specific fluorescence at all [14,15], although all these tissues contain active tyrosinase and substantial amounts of dopa. The main substance leading to a specific fluorescence of melanin-producing cells in human tissues has been shown to be 5-S-cysteinyldopa rather than dopa (for review, see reference 16). This substance is formed by the reaction of dopa and other melanin intermediates with sulfhydryl compounds [17,18].

In the present study, formaldehyde-induced fluorescence microscopy has been applied to embryonic skin and eyes, and to melanomas of xiphophorine fish, in order to find out whether this type of fluorescence can be demonstrated also in pigment cells of lower vertebrates. If this method can be used to distinguish between fish pigment cells of different degrees of differentiation, i.e., between young melanin-synthesizing cells (melanocytes) and mature inactive cells (melanophores), it might represent a valuable technique in the characterization of fish melanomas.

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MATERIALS AND METHODS

Pregnant swordtail females (*Xiphophorus helleri*) and melanoma-bearing platyfish-swordtail hybrids (*Platypoecilus maculatus/X. helleri* × *X. helleri*) were derived from stocks established at the Institute of Genetics in Giessen. The hybrids carried the gene complex *Sd* [Spotted dorsal [19] which causes melanoma formation in the dorsal fin. The material included wild-type pigmented and albino fish.

Fish embryos were isolated from the pregnant females. Three pigmented and three albino embryos in stage 20 of embryogenesis (according to Tavolga, [20]) were selected. At this developmental stage, all stages of pigment cell differentiation are present in the skin, and the cells of the pigment epithelium in the eyes have finished melanin synthesis. Melanomas were selected with regard to growth rate. Eight melanotic melanomas from wild-type pigmented fish and eight amelanotic melanomas from albino fish were used, including slowly-growing, rapidly growing, and very rapidly growing tumors. These three tumor types are known to differ from each other in the level of tyrosinase activity and in the content of incompletely differentiated pigment cells [21,22].

Embryos and melanomas were frozen in liquid nitrogen, and sections were prepared for formaldehyde-induced fluorescence as described earlier [7]. Control sections were treated in the same way, except that formaldehyde treatment was omitted.

RESULTS

Sections of pigmented embryos did not show any green-yellow fluorescence of pigment cells. The melanin-containing cells of the skin were visible as black, flattened cells immediately below the basal lamina (Fig 1*a*). At the same level and alternating with the black cells, there were cells of the same size and shape but showing a bright orange fluorescence. Both cell types were observed also in the choroid layer of the eyes. Within the eyes, the cells of the pigment epithelium were visible as black cells with fine dendrites branching into the retinal layer (Fig 1*b*). Sections of albino embryos showed the same pattern of fluorescence except for those cells corresponding to the black cells of pigmented embryos. These cells in albino embryos showed a green-yellow fluorescence which could be distinguished from the weak green autofluorescence of the tissues. In the skin, the cells with green-yellow fluorescence alternated with those of orange fluorescence (Fig 1*c*). In the eyes, a green-yellow fluorescence was observed in cells of the pigment epithelium (Fig 1*d*).

In all embryos, the size and density of black and fluorescent cells in the skin decreased from the head to the tail region. In the head, these cells were large and flattened and were present in high numbers. They showed the typical size and shape of mature pigment cells. In the trunk, most of the cells were smaller and highly dendritic, their number decreasing with increasing distance from the head. In the tail region, only a few very small dendritic cells were observed. All dendritic cells showed the typical size and shape of incompletely differentiated pigment cells.

Sections of the melanotic melanomas from pigmented fish and the amelanotic melanomas from albino fish did not show any specific fluorescence. The pigment cells of melanotic melanomas were visible as black cells, exhibiting the characteristic size and shape of melanocytes or melanophores, respectively (Fig 2*a*). In the albino melanomas, individual pigment cells could not be identified (Fig 2*b*).

DISCUSSION

In lower vertebrates, there are 3 types of pigment cells in the skin: black melanophores, red or yellow pterinophores, and reflecting iridophores. All these cells develop from a common stem cell, the neural crest-derived chromatoblast [23]. From this stem cell, the black pigment cells differentiate via unpigmented melanoblasts into melanin-synthesizing melanocytes which finally differentiate into synthetically inactive melanophores (for terminology, see reference 24).

In the skin of xiphophorine fish embryos, all stages of pigment cell differentition can be observed, while in the skin of adult fish, only mature pigment cells are visible. In certain platyfish-swordtail hybrids, however, melanocytes of a special type fail to differentiate into melanophores and, thus, give rise to the formation of malignant melanomas [25]. Most of the melanoma

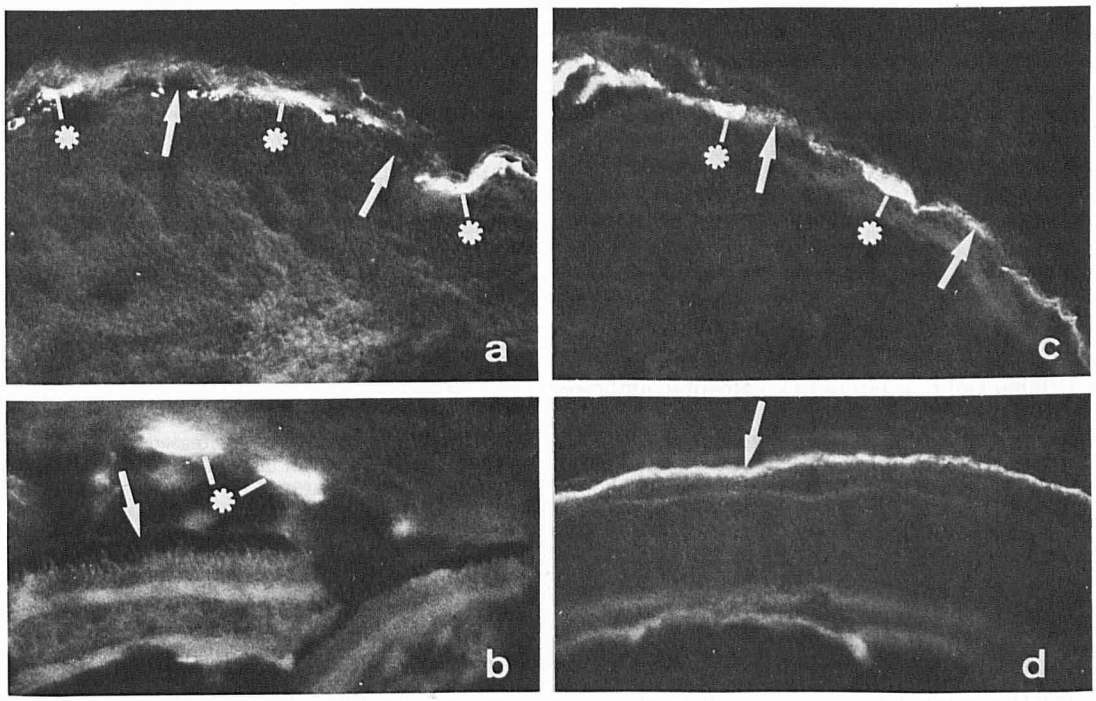

FIG 1. Sections of fish embryos, formaldehyde-induced fluorescence. *a*, Skin of the head region of a pigmented embryo. Absence of fluorescence in the black melanophores (→) but a bright organge fluorescence in the red pterinophores (*). *b*, Eye of a pigmented embryo. Absence of fluorescence in the pigment epithelium (→), bright orange fluorescence of pterinophores in the choroid layer of the eye (*). *c*, Skin of the head region of an albino embryo. Green-yellow fluorescence in those cells of the skin which correspond to the black melanophores in pigmented embryos (→) and bright organge fluorescence in the red pterinophores (*). *d*, Eye of an albino embryo. Green-yellow fluorescence in the pigment epithelium (→). (× 220).

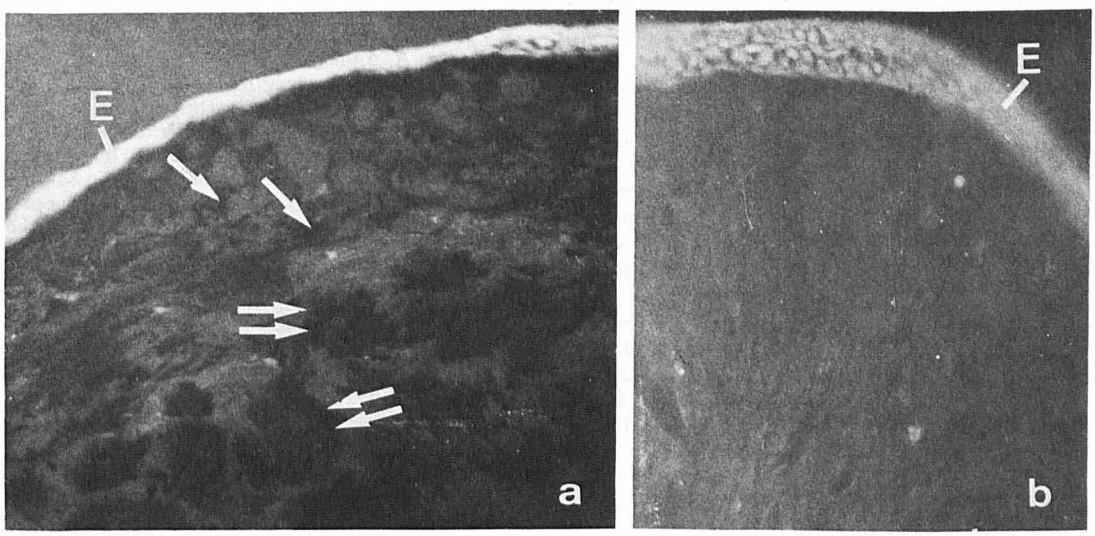

FIG 2. Sections of fish melanomas, formaldehyde-induced fluorescence. *a*, Melanotic melanoma of a pigmented fish. Absence of pigment cell-specific fluorescence in melanocytes (→) and melanophores (⇉). *b*, Amelanotic melonoma of an albino fish. No specific fluorescence in melanoma cells. *E*, epidermis (× 220).

cells continue to produce melanin in high amounts. The levels of tyrosinase activity in melanomas of different growth rates correlate with the degree of malignancy and the amount of incompletely differentiated cells present in the tumor [21,22]. If the formaldehyde-induced fluorescence microscopy, which is used to identify melanin-producing cells in human tissues, can be used to distinguish between synthetically active melanocytes and inactive melanophores, this method could become a valuable histochemical technique in the characterization of a fish melanoma with respect to its growth rate and degree of malignancy.

In the present study, however, we failed to demonstrate a specific green-yellow fluorescence in normal and malignant pigment cells of nonalbino fish. Since both the skin of embryos and the melanomas are known to contain synthetically active melanocytes [22], the failure to induce such a fluorescence in these tissues cannot be explained by the assumption that melanin precursors are lacking. The more likely interpretation is that, in melanocytes of pigmented fish, 5-S-cysteinyldopa, the substance which is responsible for formaldehyde-induced fluorescence in human melanocytes [16], is not formed, because sulfhydryl compounds which could react with dopa may not be present.

In contrast to the pigmented embryos, the albino embryos showed a green-yellow fluorescence in all cells which correspond to the black pigment cells in the skin and eyes of pigmented embryos. This agrees with the results of other authors [26] who observed formaldehyde-induced fluorescence in the ocular pigment cells of albino embryos but not of pigmented embryos from various mammals. The fluorescence in albino pigment cells of fish and mammals suggests an accumulation of substances which are very similar to or identical with 5-S-cysteinyldopa. Until now, no fluorescence has been found in albino melanoma cells, although Greenberg et al [27] have demonstrated sulfhydryl compounds in these cells. In the case of very rapidly-growing, completely amelanotic albino melanomas, the absence of fluorescence may be explained, at least in part, by the melanoblast-like, synthetically inactive state of the melanoma cells [21]. The absence of fluorescence in the more slowly-growing, partially melanotic albino melanomas is not yet understood. Perhaps, the metabolic pathways leading to melanin precursors differ in normal and malignant albino pigment cells. This view is confirmed by the fact that the cells of albino melanomas are able to produce a brown pigment, while the melanocytes of albino skin are unable to do so.

In the skin of pigmented and albino embryos, cells have been observed which show a bright orange fluorescence. Since an orange fluorescence induced by formaldehyde treatment is characteristic for certain pterines (M. Henze, personal communication), and since these cells show the typical size, shape, and location of pigment cells, they represent pterinophores.

In summary, the present study revealed that formaldehyde-induced fluorescence microscopy, which has become an important technique in the diagnosis of human pigmentary disorders [2–8], cannot be used to identify pigment cells in nonalbino fish. Taken into account that fish melanocytes synthesize eumelanin, while human melanocytes may produce various amounts of phaeomelanin instead of or in addition to eumelanin [28,29], the failure to demonstrate a specific fluorescence in melanin-producing cells of fish may be due to the absence of 5-S-cysteinyldopa which is the key intermediate in the formation of phaeomelanin [18]. We propose the same explanation for nonalbino pigment cells of various mammals [14,15,26], since these cells are thought to synthesize eumelanin, the key intermediate of which is 5,6-dihydroxyindole [28,30].

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